Claims

1. A water-soluble aggregate of insulin derivatives, characterised by having a size larger than aldolase as determined in a gel filtration system as specified above.

2. An aggregate according to claim 1, characterised by having a size larger than ferritin as determined in a gel filtration system as specified above.

3. An aggregate according to claim 1 having an apparent volume corresponding to a K_{AV} value of less than 0.32, preferably less than 0.20, as determined by gel filtration using a Sephacryl® S-300 HR gel.

4. An aggregate according to claim 1 having an apparent volume corresponding to a K_{AV} value of less than 0.50, preferably less than 0.40, as determined by gel filtration using a Superose® 6HR gel.

5. An aggregate according to any one of claims 1 to 4, characterised by being soluble at a pH in the range of 6.8 to 8.5.

6. An aggregate according to any one of claims 1 to 5, composed essentially of hexameric subunits of insulin derivatives.

7. An aggregate according to claim 6, composed of at least 4, preferably 5 to 50, more preferably 5 to 200 aggregated hexameric subunits.

8. An aggregate according to any one of claims 1 to 7, comprising at least 2 zinc ions, preferably 2 to 5 zinc ions, more preferably 2 to 3 zinc ions, per 6 molecules of insulin derivative.

9. An aggregate according to any one of claims 1 to 8, comprising at least 3 molecules of a phenolic compound per 6 molecules of insulin derivative.

10. An aggregate according to any one of claims 1 to 9, having a disappearance half-time after subcutaneous injection in humans as long as or longer than that of a human insulin NPH preparation.

11. An aggregate according to any one of claims 1 to 10, in which the insulin derivative has an albumin binding which is lower than that of $Lys^{B29}(N^{\varepsilon}$-tetradecanoyl) des(B30)

human insulin.

12. An aggregate according to any one of claims 1 to 11, in which the insulin derivative contains only substitutions relative to human insulin.

13. An aggregate according to any one of claims 1 to 12, in which the residues B24-B30 of the B-chain of the insulin derivative is the sequence Phe-X-X-X-X-X-X, where each X independently represents any amino acid or a deletion, at least one X being a N^{ε}-substituted lysine residue.

14. An aggregate according to any one of claims 1 to 13, in which the residues B25-B30 of the B-chain of the insulin derivative is the sequence Phe-X-X-X-X-X, where each X independently represents any amino acid or a deletion, at least one X being a N^{ε}-substituted lysine residue.

15. An aggregate according to any one of claims 1 to 14, in which the residues B26-B30 of the B-chain of the insulin derivative is the sequence Tyr-X-X-X-X, where each X independently represents any amino acid or a deletion, at least one X being a N^{ε}-substituted lysine residue.

16. An aggregate according to any one of claims 1 to 15, in which the residues B27-B30 of the B-chain of the insulin derivative is the sequence Thr-X-X-X, where each X independently represents any amino acid or a deletion, at least one X being a N^{ε}-substituted lysine residue.

17. An aggregate according to any one of claims 1 to 16, in which the residues B28-B30 of the B-chain of the insulin derivative is the sequence Pro-X-X, where each X independently represents any amino acid or a deletion, at least one X being a N^{ε}-substituted lysine residue.

18. An aggregate according to any one of claims 1 to 17, in which the residues B29-B30 of the B-chain of the insulin derivative is the sequence Lys-X, where X represents any amino acid or a deletion.

19. An aggregate according to any one of claims 13 to 18, in which each X independently is selected from the following group of amino acids: Phe, Tyr, Thr, Ser, Pro, Lys, Gly, Ala, Glu, Asp, Gln, His or is deleted.

20. An aggregate according to any one of claims 13 to 19, in which X in position B25 is selected from the following group of amino acids: Tyr, Phe, His, Gly or is deleted.

21. An aggregate according to any one of claims 13 to 20, in which X in position B26 is selected from the following group of amino acids: Thr, Ala, Phe, Tyr or is deleted.

22. An aggregate according to any one of claims 13 to 21, in which X in position B27 is selected from the following group of amino acids: Glu, Gln, Lys, Pro, Gly, Ala, Ser, Thr or is deleted.

23. An aggregate according to any one of claims 13 to 22, in which X in position B28 is selected from the following group of amino acids: Asp, Glu, Gly, Ala, Lys, Pro or is deleted.

24. An aggregate according to any one of claims 13 to 23, in which X in position B29 is selected from the following group of amino acids: Asp, Glu, Gly, Ala, Pro, Thr, Lys or is deleted.

25. An aggregate according to any one of claims 13 to 24, in which X in position B30 is selected from the following group of amino acids: Lys, Ala, Ser, Thr or is deleted.

26. An aggregate according to any one of claims 13 to 25, in which the amino acid in position A21 is selected from group consisting of Ala, Asn, Gln, Glu, Gly and Ser.

27. An aggregate according to any one of claims 13 to 26, in which the amino acid in position B1 is selected from Asp, Thr, Asn, Ser, Pro, Gln, Gly, Phe or is deleted.

28. An aggregate according to any one of claims 13 to 27, in which the amino acid in position B2 is selected from Glu, Pro, Asp, Ala and Val.

29. An aggregate according to any one of claims 13 to 28, in which the amino acid in position B3 is selected from the group consisting of Asn, Gln, Glu, Asp, Ala and Thr.

30. An aggregate according to any one of claims 13 to 29 in which the amino acid in position B13 is Glu or Gln.

31. An aggregate according to any one of claims 13 to 30 in which the amino acid in each of the positions A1-A20, B4-B12, and B14-B24 is the corresponding amino acid in human insulin.

32. An aggregate according to any one of claims 13 to 31 in which the insulin derivative contains only one lipophilic substituent.

33. An aggregate according to any one of claims 13 to 31 in which the total number of different amino acids between the insulin derivative and human insulin does not exceed six, preferably is five, more preferably is four, even more preferably is three, even more preferably is two, and most preferably is one.

34. An aggregate according to any one of claims 13 to 31, in which the substituent at the lysine residue is a lipophilic group containing from 6 to 40 carbon atoms.

35. An aggregate according to claim 34, in which the substituent is an acyl group having from 6 to 40, preferably 12 to 36, carbon atoms.

36. An aggregate according to claim 35, in which the acyl group is CH_3-$(CH_2)_n$-CO-, where $4 \leq n \leq 38$.

37. An aggregate according to claim 35, in which the acyl group is (COOH)-$(CH_2)_n$-CO-, where $4 \leq n \leq 38$.

38. An aggregate according to claim 35, in which the acyl group is $(NH_2$-CO)-$(CH_2)_n$-CO-, where $4 \leq n \leq 38$.

39. An aggregate according to claim 35, in which the acyl group is HO-$(CH_2)_n$-CO-, where $4 \leq n \leq 38$.

40. An aggregate according to claim 35, in which the lipophilic substituent is 5-α lithocholic acid or 5-β lithocholic acid.

41. An aggregate according to claim 35, in which the lipophilic substituent is 5-α or 5-β isomers of cholic acid, hyocholic acid, deoxycholic acid, chenodeoxycholic acid, ursodeoxycholic acid, hyodeoxycholic acid or cholanic acid.

42. An aggregate according to claim 35, in which the lipophilic substituent is a 5-α or 5-β isomer of dehydrolithocholic acid.

43. An aggregate according to claim 35, in which the lipophilic substituent is fusidic acid, a fusidic acid derivative or glycyrrhetinic acid.

44. An aggregate according to claims 35-43, in which the acyl group is linked to the lysine residue using an amino acid as linker.

45. An aggregate according to claim 44, in which the amino acid link is α-glutamyl or γ-glutamyl bonded or β- or α-aspartyl bonded.

46. An aggregate according to claim 44, in which the amino acid link is γ-aminobutanoyl bonded, β-alanyl bonded, α-amido-γ-glutamyl bonded, or α-amido-β-aspartyl bonded.

47. A novel insulin derivative capable of forming aggregates according to any one of the preceding claims.

48. A pharmaceutical preparation comprising an aggregate of insulin derivatives according to any one of claims 1 to 47.

49. A pharmaceutical preparation according to claim 48, comprising aggregates according to any one of claims 1 to 33, a substantial fraction of which, preferably more than 75%, has a larger size than aldolase as determined by gel filtration using the medium of the preparation as eluent.

50. A pharmaceutical preparation comprising both aggregating insulin derivatives and rapid acting insulin analogues, the latter preferably being human insulin or Asp^{B28} human insulin, $Lys^{B28}Pro^{B29}$ human insulin or des(B30) human insulin.

51. A pharmaceutical preparation according to claim 50, in which the molar ratio of aggregating insulin to rapid acting insulin is 90:10 to 10:90.

52. A pharmaceutical preparation according to any one of claims 48 to 51, comprising an agent which increases the viscosity, preferably polyethylene glycol, polypropylene glycol, copolymers thereof, dextrans and/or polylactides.

53. A pharmaceutical preparation according to any one of claims 48 to 52, comprising a buffer substance, preferably a TRIS, phosphate or glycine buffer.

54. A pharmaceutical preparation according to any one of claims 48 to 53, comprising a zwitterionic buffer substance, preferably glycylglycine.

55. A pharmaceutical preparation according to any one of claims 48 to 54, comprising

an isotonic agent, preferably NaCl, glycerol, mannitol and/or lactose.

56. A pharmaceutical preparation according to any one of claims 48 to 55, comprising Na^+ ions in a concentration of 10 to 150 mM.

57. A pharmaceutical preparation according to any one of claims 48 to 56, comprising phenol and/or m-cresol as preservatives.

58. A pharmaceutical preparation containing 0.1-2 mM of an insulin derivative according to claim 31, 0.3-0.9% Zn (w/w relative to insulin derivative), and phenolic compounds like phenol or m-cresol or mixtures hereof in a total concentration of 5-50 mM, and Na^+ ions in a concentration of 10 mM to 150 mM.

59. A method of treating diabetes mellitus comprising administering to a person in need of such treatment an effective amount of water-soluble aggregates of insulin derivatives according to any one of the claims 1 to 46.

60. A method of treating diabetes mellitus comprising administering to a person in need of such treatment an effective amount an insulin derivative according to claim 47, capable of forming water-soluble aggregates upon subcutaneous injection.